

Structural and gelling properties of very low methoxyl pectin produced by an alkali-treatment

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Abstract Very low methoxyl pectin (VLMP) has different physical and rheological properties compared to high and low methoxyl pectins (HMP and LMP). In this study, we produced LMP and VLMP by alkaline de-esterification, and investigated the structural and textural properties. Apple peel pectin was kept at pH 12 using 5.0 M NaOH solution for 3 and 24 h to produce LMP and VLMP, respectively. The molecular weight was decreased due to the removal of an esterified group in the pectin backbones by the alkali treatment, and the VLMP showed a higher calcium ion sensitivity which leads to the production of the gel with increased hardness. The result clearly showed that VLMP has the potential to improve the texture and stability in food products depending on their degree of esterification, and this result can be applied as a functional ingredient in food industrial area application to enhance the current commercial pectins.

Keywords: de-esterification, very low methoxyl pectin, calcium ion sensitivity, pectin gel, alkali-treatment

Introduction

Pectins are major components in the primary cell wall of plants, and mainly consist of more than 65% of galacturonic acids (GalUA) and neutral sugars such as rhamnose, arabinose, glucose, and xylose which are glycosidic-linked in GalUA backbone (Kravtchenko et al., 1992). Pectins have been widely used as food ingredients such as stabilizers in dairy foods, emulsifiers in oily foods, thickeners in sauces, and gelling agents in jams (Laurent and Boulenguer, 2003; Leroux et al., 2003). Furthermore, applications of pectin in cosmetic, pharmaceutical and food industrial areas steadily increased as the health functionalities have been identified such as prevention of gastrointestinal diseases, skin anti-aging, and anti-tumor activity (Hayashi et al., 2000; Lebreton-Decoster et al., 2011). Pectins can be classified into two groups by the degree of their esterification (DE) values, high-methoxyl pectin (HMP) and low-methoxyl pectin (LMP) based on the degree of methyl esterified GalUA groups in the pectin molecules (El-Nawawi and Heikal, 1995). The ratio of methyl esterified GalUA is higher than 50% in HMP, while LMP contains less than the half of methyl ester groups. In general, commercial LMP is obtained by a de-esterification processing of HMP with acidic, alkaline, and enzymatic treatments (Hotchkiss et al., 2002; Ralet et al., 2001).

As HMP and LMP have different gelation properties, there are different applications in the food industry: HMP requires large

amounts of sucrose at low pH condition (<3.5) for gel production, whereas LMP can produce gels without additional sugars in the presence of divalent cations (Gadalla et al., 2016). LMP is less sensitive to pH compared to HMP during the gelling process (Hotchkiss et al., 2002), and thus the LMP production is an important process to enhance the pectin applications as a functional ingredient for low-calorie and low-sugar foods (Broomes and Badrie, 2010; Sila et al., 2009). Especially, the very low methoxyl pectin (VLMP) with lower DE values (<20) has different physical characteristics compared to typical LMPs such as the enhanced properties of gel formation by calcium ion (Ström et al., 2007) or difference in released aroma constituents (Kim et al., 2016).

To produce VLMP, previous researchers widely used the pectin methyltransferase (PME) to produce LMPs (Kim et al., 2014). However, some pectin structures have been destroyed depending on the type of PME or it was difficult to commercially producing by PME treatment because they mainly used directly refined PME or using reagent grade PME (Hua et al., 2018). Therefore, we attempted an alkaline de-esterification that can be relatively decreased structural damage and increased the possibility of industrial use. Furthermore, as the alkali processing time was adjusted to create VLMP, we analyzed physical properties and identified the food applicability of VLMP as a food stabilizer, which is differentiated from the existing commercial LMPs.

Materials and Methods

Materials

Commercial HM and LM pectins were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and CP Kelco (Chicago, IL, USA), respectively. The pectin from apple peel waste was extracted based on the previous research (Cho et al., 2019), and

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applied as a source of raw pectins for de-esterification in the current research. The pectin sample was dialyzed against distilled water for 24 h using a dialysis membrane (MWCO: 3,500 Spectrum Laboratories Inc., Rancho Dominguez, CA, USA). All other chemical reagents used in the experiments were analytical grade.

Alkaline de-esterification using sodium hydroxide

Pectin solutions (1.0%, w/v) were adjusted to alkaline conditions (pH 11, 12 and 13) using 5.0 M sodium hydroxide (NaOH). The pH changes in pectin solutions were measured every 3 h, and adjusted by NaOH solution with stirring. After 24 h, the pH level of pectin solutions was decreased to 5.5 using 0.1 N hydrochloric acid solution (HCl) for finishing de-esterification process by alkaline treatment. Then, three times the volume of isopropyl alcohol (95%, v/v) was added to precipitate the de-esterified pectin, and the precipitation was dialyzed. Dialyzed pectins solutions were lyophilized for further experiments (Hotchkiss et al., 2002).

Determination of degree of esterification value

For determination of GalUA, 0.4 mL of the pectin solution (0.01%, w/v), 40 μ L of 4 M sulfamate solution, and 2.5 mL of 75 mM sodium tetraborate in concentrated sulfuric acid were mixed. Then, the solution was heated for 15 min and immediately cool down to room temperature. Finally, 80 μ L of *m*-hydroxydiphenyl dissolved in 0.5% NaOH solution (0.15%, w/v) was added into the mixture, and the change of absorbance was read at 520 nm within 5 min (Filisetti-Cozzi and Carpita, 1991). Potassium hydroxide solution (1.0 N, 25 mL) was added to the prepared pectin solutions (0.1%, w/v, 25 mL) to measure the amount of released methanol content. Alcohol oxidase (1 unit/mL, Sigma-Aldrich Chemical Co.) was added to 1 mL of prepared mixture and incubated at 25°C for 15 min. Then, 2,4-pectanedione in 2.0 M ammonium acetate and 0.05 M acetic acid reagent (2 mL) was added, followed by incubation at 60°C for 15 min. The absorbance of reactants was measured at 412 nm, and the DE value was calculated by the amount of methanol content (% w/v) was divided by the sum of GalUA (% w/v) and methanol content (% w/v) (Klavons and Bennett, 1986).

$$\text{DE value (\%)} = \frac{\text{Methanol (\%)}}{\text{GalUA (\%)} + \text{Methanol (\%)}} \times 100$$

Measurement of molecular weight distribution after the de-esterification

To determine the molecular weight of de-esterified pectin samples, the high-performance size-exclusion chromatography connected with multiple-angle laser light scattering (MALS) and refractive index (RI) detectors (Wyatt Technology Corp., Santa Barbara, CA, USA) were used. Individual pectin samples were dissolved in 50 mM sodium nitrate (0.2%, w/v), and the filtered-pectin sample (100 μ L) was injected into Shodex OH-806 and 804 HQ coupled columns (Showa Denko, Tokyo, Japan). For the mobile phase, a 50 mM sodium nitrate solution was used at 50°C with a flow rate of 0.4 mL/min. The weight-average molecular

weight (M_w) of de-esterified pectins was calculated using ASTRA™ software (Version 5.3.4, Wyatt Technology Corp.) with Zimm plots (dn/dc value: 0.146) (Fishman et al., 2006).

Viscosity measurement of alkali-modified pectin solution

The prepared pectin solutions (0.5 mL, 1%, w/v) were placed on the center of a Peltier plate of a dynamic rheometer (DHR1, TA Instruments, New Castle, DE), and the steady shear was measured by a plate and plate geometry system (4 cm diameter and 500 μ m) over a shear rate of 0.1 to 100 s^{-1} at 25°C. The consistency index (K), flow behavior index (n), and apparent viscosity (at 100 s^{-1}) were calculated using the Power-Law model (Doi, 1981).

Calcium ion sensitivity analysis of de-esterified pectins

To determine the calcium ion sensitivity of de-esterified pectin, the previous method was slightly modified (Gerrish, 1998). Pectin solutions (0.2 g/10 mL) were heated in the water bath at 70°C until completely dissolved. Then, the pH was adjusted to 4.0 and chilled isopropyl alcohol (IPA; 80%, v/v, 20 mL) was added. The precipitant was collected by centrifugation at 8000 \times g for 30 min at 10°C and it was washed twice with 60% IPA. The washed tubes were then dried under a vacuum and weighed. The weight of the pectin precipitant was divided by the initial weight of pectin added to the tube, and this value was designated A. CaCl₂ (30 mM) in 8% IPA (10 mL) was added to the second of the duplicate tubes. These tubes were incubated for 24 h at 25°C with occasional vortexing. The produced gel was collected and weighed in same way as the above method (designated B). The calcium ion sensitivity was determined by dividing B value by A value.

Analysis of pectin gel properties

Sucrose was added into the pectin solution (final conc.: 15%, w/v), and then calcium phosphate (0.1%, w/v) and glucono- δ -lactone (GDL; 0.45%, w/v) were added into the solution after complete dissolution of the pectin gel production. To measure the hardness of the pectin gels, they were kept at room temperature before the hardness analysis. A hardness analysis was performed using TA-XT2 Texture Analyzer (Stable Micro systems, Surrey, UK). A sample of pectin gel was laid on a plate and was compressed at 1 mm/s with a 50 mm diameter cylinder aluminum probe and until compression was 40% of the initial height of samples.

Statistical analysis

All experiment data were statistically analyzed using SAS software system (version 9.4, SAS Institute, Cary, NC). The significant differences ($p < 0.05$) between means were determined using analysis of variance (ANOVA) with Tukey tests.

Results and Discussion

The change of degree of esterification by alkaline treatment

The DE values of the apple pectin with de-esterification at pH 12 were calculated after 3 and 24 h (Table 1). The DE values of 3 h- and 24 h-treated apple pectins were showed 23.8% and 17.3%, respectively. The result confirmed that the pectin structure

Table 1. The degree of esterification (DE) values of alkali-treated apple pectin according to the treatment time

	0 h	3 h	24 h
DE values	58.6±1.1 ^{a1)}	23.8±3.2 ^b	17.3±6.5 ^{bc}

¹⁾Mean values of duplicate measurements; values in row with different letters in superscripts for each pectin sample are significantly different ($p<0.05$)

Table 2. Average molecular mass of alkaline modified pectin by HPSEC-MALS-RI analysis

Type of pectin	Weight-average molecular mass ¹⁾ ($M_w \times 10^{-5}$ g/mol)	M_w/M_n ²⁾
Control ³⁾	5.41±0.41 ^{ab}	2.25±0.63 ^a
HMP	6.29±0.54 ^a	1.79±0.25 ^a
LMP	4.91±0.13 ^b	1.87±0.57 ^a
VLMP	3.66±0.01 ^c	2.16±0.42 ^a

¹⁾Values with different letters in each column were significantly different ($p<0.05$) based on ANOVA and Tukey's tests

²⁾Polydispersity index

³⁾Control, commercial high methoxyl pectin from apple (Sigma-Aldrich); HMP, high methoxyl pectin; LMP, low methoxyl pectin; and VLMP, very low methoxyl pectin

can be changed from HMP to LMP by alkali treatment, and VLMP was produced at 24 h that has the longest alkali reaction time with the lowest DE value. Furthermore, VLMP with much lower DE value than commercial LM pectin can be effectively produced by the alkali treatment. A previous study described that the VLMP has different physiochemical characteristics such as calcium ion sensitivity and gelation properties compared to HMP and LMP (Kim et al., 2016). Thus, it is expected that the VLMP from apple in this study is worth proving with different physical properties compared to commercial pectins.

Molecular weights and fluid properties of alkali-treated pectins

The molecular averages of commercial pectin, apple-peel pectin which is classified as a HMP, and alkaline modified LMP and VLMP were shown with polydispersity in Table 2. The apple-peel HMP extracted by citric acid has a higher molecular weight

compared to commercial pectin (6.29×10^{-5} g/mol). On the other hand, two alkali-treated LMP and VLMP (M_w 4.91×10^{-5} g/mol and 3.66×10^{-5} g/mol, respectively) had lower M_w than HMP. These results can explain that the homogalacturonan backbone α -(1,4)-glycosidic linkage was degraded by β -elimination with alkali de-esterification of pectins, and this property lead to decrease of molecular weight (Selvendran et al., 1985). Polydispersity index ($=M_w/M_n$) refers molecular weight distribution of polymers, thus smaller polydispersity index polymer has narrow molecular weight distribution (Peebles, 1971). As shown in Table 2, the polydispersity index of all pectin samples showed no significant difference after alkali de-esterification (1.79-2.25). This result clearly demonstrated that the depolymerization of pectin structures that were caused by alkali treatment took place in both the low and high molecular areas (Yapo et al., 2007).

Table 3 shows the fluid properties of pectins that have different DE values using Power-Law model parameters, and the calculation of the apparent viscosities was performed at the shear rate of 100 s^{-1} in the same way. The higher molecular weight pectin has significantly higher flow consistency and apparent viscosity. The flow consistency of HMP was $18.77 \text{ mPa} \cdot \text{s}$ and the apparent viscosity was $12.81 \text{ mPa} \cdot \text{s}$ which has higher values compared to commercial pectin as a control. On the other hand, the flow consistency and the apparent viscosity of the alkali-treated pectins with low DE values were decreased which can be explained by their molecular weight and viscosity (Kar and Arslan, 1999). As the alkali exposure time increased, the β -elimination of pectin structures occurred more frequently. Thus, the hydrophobic interaction of the pectin was weakened in aqueous pectin solutions (Yoo et al., 2012). In general, the β -elimination of pectin is an ordinary phenomenon during the chemical de-esterification such as acid and alkali treatments (Renard and Thibault, 1996). Therefore, the LMPs in this study also could not avoid a reduction in molecular weight and viscosity. Nevertheless, LMPs have higher calcium ion sensitivity than HMP and flexible gel producing condition (Hotchkiss et al., 2002). Thus, the depolymerized LMPs still can be applied to other applications compared to HMPs. In the flow behavior index (n) values, all pectin samples exhibited ranging from 0.92 to 0.99. Fluids with a flow behavior value of less than 1.0 is called a pseudoplastic fluid ($0.4 \leq n \leq 1.0$), which decreased in shear stress as the shear rate increase (Boger, 1977).

Table 3. Power-Law-model¹⁾ parameters and apparent viscosities of pectin at differential degree of esterification

	K ²⁾ ($\text{mPa} \cdot \text{s}^n$)	n	r^2	η ($\text{mPa} \cdot \text{s}$)
Control ³⁾	12.37±0.00 ^b	0.97±0.02 ^{ab}	1.00	10.66±0.36 ^b
HMP	18.77±0.00 ^a	0.92±0.00 ^b	1.00	12.81±0.20 ^a
LMP	4.87±0.00 ^c	0.99±0.01 ^a	1.00	4.58±0.04 ^c
VLMP	4.27±0.00 ^c	0.98±0.01 ^a	1.00	3.91±0.11 ^d

¹⁾Means of triplicate, Values with different letters of a given pectins were significantly different from each other ($p<0.05$) based on ANOVA and Tukey's tests.

²⁾ K , flow consistency index; n , flow behavior index; η , apparent viscosity at 25°C and 100 s^{-1} .

³⁾Control, commercial high methoxyl pectin from apple (Sigma-Aldrich); HMP, high methoxyl pectin; LMP, low methoxyl pectin; and VLMP, very low methoxyl pectin.

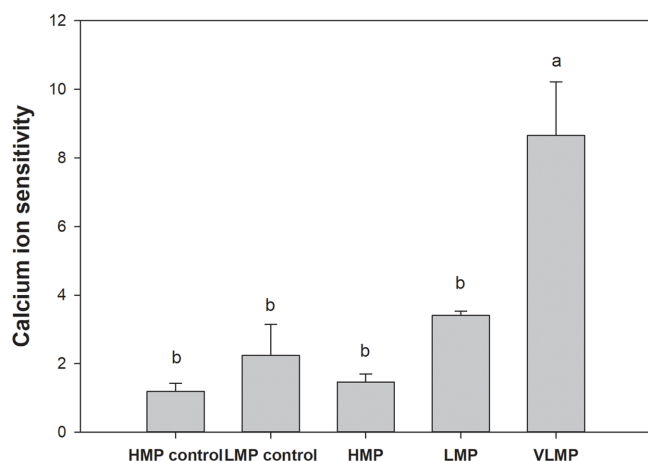


Fig. 1. Calcium ion sensitivity of differential DE value pectins. Means of duplicate. Values with different letters of a given pectins were significantly different from each other ($p < 0.05$) based on ANOVA and Tukey's tests. HMP control, high methoxyl commercial pectin; LMP control, low methoxyl commercial pectin; HMP, high methoxyl pectin; LMP, low methoxyl pectin; and VLMP, very low methoxyl pectin.

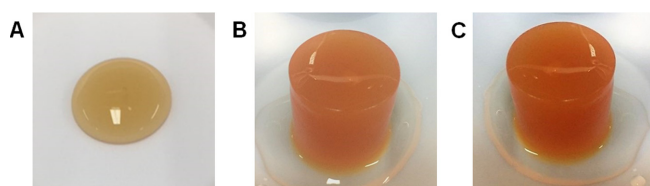


Fig. 2. Shapes of pectin gel made under the same condition. (A) high methoxyl pectin; (B) low methoxyl pectin; and (C) very low methoxyl pectin.

Calcium ion sensitivity of alkali de-esterified pectins

The calcium ion sensitivity is a relative measurement for the gel production whether or not it can be produced without the addition of sucrose by blocking of de-esterified GalUA groups in pectin (Joye and Luzio, 2000). Pectins with higher calcium ion sensitivity showed more gelation property because of the calcium ion and free carboxyl groups in the pectins' structure by ionic interactions of polyvalent cations (Rinaudo, 1996). Thus, the pectin with the higher calcium ion sensitivity can be applied in dairy drinks, low-sugar jams or jellies (Joye and Luzio, 2000). Figure 1 shows the calcium ion sensitivity of the pectins depending on their DE values. As the DE value of alkali-treated pectin decreased, it clearly had higher calcium ion sensitivity. Especially, the VLMP had the highest calcium ion sensitivity (8.66) among other pectin samples. The significance of these results suggested that the VLMP has a potential to apply to dairy food products containing calcium as a gelling agent or a stabilizer.

Characteristic effects of alkaline modified pectins on gel texture

The texture properties of pectin gel such as hardness and syneresis were greatly influenced by GDL concentration in the presence of calcium ions. Therefore, all the differential DE values

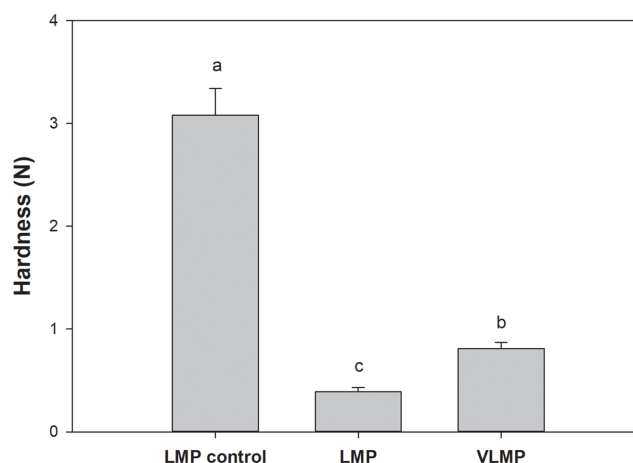


Fig. 3. The gel hardness of low methoxyl pectins produced by alkali treatment from apple peel. LMP control, low methoxyl commercial pectin; LMP, low methoxyl pectin; and VLMP, very low methoxyl pectin. Values with different letters in each graph were significantly different ($p < 0.05$) based on ANOVA and Tukey's tests.

of pectin gel samples were produced under the same GDL conditions based on a previous study (Yoo et al., 2009). Figure 2 showed the shape of pectin gels with different DE values made with sucrose, calcium ion, and GDL. LMP and VLMP were formed in the shape of hard gels, however the HMP showed only high viscosity fluid with no formatting gel due to their different gelling mechanism compared to LMP. It is expected that a higher amount of sucrose is required for the gelation of HMP (Oakenfull, 1991). However, a moderate amount (about 10-30%) of sucrose can increase the stability of LMP during gelling production as additional hydroxyl groups from sucrose promote the structuralization of cross-linking junction zones (egg-box model) with a hydrogen bond (Fu and Rao, 2001). But excessive amounts of sucrose is unnecessary in the formation of gel in LMPs which may lead to a decrease in the gel quality (Padival et al., 1979). Therefore, further studies were able to accurately compare gel characteristics depending on the DE value by controlling the conditions of gel production for the LMPs.

When the pectin gels were made of the same constituent, the lower DE values of the pectins produced harder gels (Fig. 3). The hardness of VLMP was 0.81 N, which was almost two times higher than LMP (0.39 N). Also, the hardness and calcium ion sensitivity of individual pectins clearly showed the proportional relationship (Fig. 1), which is corresponding to previous research (Kim et al., 2008). However, the hardness of alkali treat LMPs was lower than the commercial LMP as a control (3.08 N). The reason may be explained by a standardized process of commercial pectin production to manufacture highly purified extracted pectins with stabilizers (e.g., sucrose) in food industries (Rolin, 2002).

Conclusions

In this research, LMP and VLMP were produced from HMP by the alkali treatment via the de-esterification process, and the LMPs have the potential to improve the texture and stability in food

products depending on their DE values. Thus, DE value-controlled pectins can be applied as a functional ingredient in food industrial areas (e.g., acidic drinks and yogurt products) to enhance the rheological properties such as viscosity or thickening agents as compared to commercial pectins.

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